# EFFECT OF INDOMETHACIN ON *SALMONELLA TYPHIMURIUM* AND CHOLERA TOXIN-INDUCED FLUID ACCUMULATION IN PIG JEJUNUM

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## ABSTRACT

We have investigated the influence of prostaglandins (PGs) on the *Salmonella typhimurium* (*St*) and cholera toxin (CT)-induced fluid accumulation in jejunum of 9-10 weeks (18-20 kg) old female pigs. *St* ($10^{10}$ colony forming units) and CT (56 $\mu$g) were instilled in separated tied-off loops for 8 hours. Before instillation, and again after 4 hours, pigs were given an intravenous dose of the cyclooxygenase and prostaglandin synthesis inhibitor, indomethacin, or saline alone (control). In the *St*-loops indomethacin had no effect on the fluid accumulation, $9.1 \pm 1.3$ vs $8.2 \pm 0.5$ mg fluid/mg loop dry weight, whereas in CT-loops, indomethacin reduced fluid accumulation significantly from $35 \pm 2$ to $22 \pm 3$ mg fluid/mg loop dry weight ($P < 0.05$). Our preliminary results indicate that PGs are not involved in *St*-induced fluid accumulation in pig jejunum, which is in contrast to the case in CT-induced fluid accumulation.

## INTRODUCTION

The pathophysiological mechanisms behind the *St*-induced diarrhoea is incompletely understood but seem to involve a CT-like enterotoxin (Duebbert and Peterson, 1985). CT induces intestinal secretion by direct and indirect stimulation of the enterocytes. The direct pathway induces $Cl^-$ secretion by increasing intracellular cAMP, whereas the indirect pathway includes release of 5-hydroxytryptamine (5-HT) from the enterochromaffine cells, which in turn initiates a secretory reflex arc in the enteric nervous system (Hansen and Skadhauge, 1997). In addition, prostaglandin subtype $E_2$ ($PGE_2$) has been reported to be involved in fluid secretion induced by CT and 5-HT in porcine intestinal loops (Hansen *et al.*, 1994; Grøndahl *et al., unpub.*). In order to investigate whether PGs also are involved in the *St*-induced fluid accumulation we investigated the effect of intravenously applied indomethacin.

## MATERIALS AND METHODS

18-20 kg (9-10-wks) female pigs (Danish Landrace/Yorkshire crossbred), all postweaned and on commercial standard diet were used. The animals were fasted overnight with free access to water containing D-glucose (55 g/l). Immediately before the experiment the pig was sedated with Sedaperone (5 mg/kg, Janssen Pharmaceuticals) injected intramuscularily. General anesthesia was maintained by α-Chloralose (15 mg in 100 ml saline, KVL Pharmacy) intravenously every 2 hours in a bolus. The pig was connected to a semiclosed circle anaesthetic machine (Dameca) with an artificial controlled respiration, and monitored by an

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ECG. The rectal temperature was monitored, and kept at 38°C using a heated mattress. The partial pressure of the blood gases and pH was measured from arterial blood samples, and all values were within normal range during the experiment. After the experiment the animal was eutanized by Pentobarbital (130 mg/kg, KVL Pharmacy) intracardially.

*Experiments:* Ligated loops were prepared in jejunum, 30 cm distal to the ligament of Treitz. Loops with a length of 20 cm, departed with 3 cm, were left unfilled, or instilled with 56 $\mu$g CT (Sigma) or living *St* ($10^{10}$ colony forming units) in 10 ml Argenzio-4 (Arg-4) solution (Argenzio, 1980). A loop instilled with 10 ml Arg-4 solution was prepared as a control for the ability of the intestinal tissue to absorb fluid. All loops were removed after 8 hours. To ensure an effective dose of indomethacin 2.5 mg/kg (Confortid, Dumex) was injected intravenously immediately before instillation, and again after 4 hours. Control pigs were given saline. Half-life of Confortid in human plasma is 4-11 hours (lægemiddelkataloget® 1995, Denmark). After removal, the loops were weighed immediately with (T), and without the fluid content (V), and then dried at 80°C to constant weight (t). Thus, fluid accumulation (remaining fluid) in the loop was calculated from the ratio (T-V)/t.

*Statistics:* Results are presented as means ± S.E.M. *N* and *n* is number of animals and loops, respectively. Data was analysed with Student's *t*-test for unpaired data. $P<0.05$ was considered significant.

## RESULTS AND DISCUSSION

In the present study we investigated the *in vivo* effect of indomethacin on the CT and *St*-induced fluid accumulation, measured as the remaining fluid in tied-off loops after 8 hours, in jejunum of pigs. Unfilled and Arg-4 loops were practically empty indicating that there was no unstimulated fluid accumulation or impaired absorption in the experimental groups (Table). In CT-loops, the cyclooxygenase and prostaglandin synthesis inhibitor, indomethacin, reduced fluid accumulation significantly, while indomethacin had no effect on the fluid accumulation in *St*-loops (Table). Different results have been observed in other species. Fluid accumulation in rat jejunum has been shown to be induced by $PGE_2$, which could be diminished by nerve blockers, indicating an action on secretory reflexes in the enteric nervous system (Brunsson *et al.*, 1987). In rabbit, indomethacin reduced *St*-induced fluid accumulation in ileum (Duebbert and Peterson, 1985), and in *St*-infected rhesus monkeys, indomethacin reversed net secretion to net absorption in ileum and colon (Giannella *et al.*, 1977). The varied results might be due to 1) the choice of *St*-bacterial strain, and thus to the synthesis of different toxins, and the ability to invade the intestinal epithelium, 2) segmental differences in the intestine, and 3) the administered indomethacin doses. In the present study, we administered a normal dose of indomethacin in order to avoid side effects that might occur in the higher pharmacological doses, *e.g.* ulcer, crypt losses and increased mitotic activity (Ettarh and Carr, 1996).

Our preliminary results indicate that PGs might not be involved in *St*-induced fluid accumulation in pig jejunum, which is in contrast to the CT-induced secretion. Other mediators and signal transduction pathways may thus be involved in *St*-induced fluid accumulation.

Table. Intestinal fluid accumulation in pig jejunum.

| Pig experiment | Loop (mg fluid/mg loop dry weight) | | | |
|---|---|---|---|---|
| | Unfilled | Argenzio-4 | Cholera toxin | *Salmonella* |
| Control ($N$=3) | 0.4±0.3 ($n$=3) | 0.5±0.1 ($n$=3) | 35±2 ($n$=3) | 9.1±1.3 ($n$=9) |
| Indomethacin ($N$=4) | 0.4±0.1 ($n$=4) | 0.5±0.1 ($n$=4) | 22±3[a] ($n$=4) | 8.2±0.5 ($n$=12) |

*Salmonella typhimurium* ($10^{10}$ colony forming units) and cholera toxin (56 μg) in 10 ml Argenzio-4 solution were instilled in tied-off loops for 8 hours. Values are means ± S.E.M. Significance of difference from control values: [a] $P<0.05$. $N$ and $n$ is the number of animals and loops, respectively.

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